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     (FILE 'HOME' ENTERED AT 12:34:17 ON 17 MAR 2003)

     FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 12:35:09
     ON 17 MAR 2003
L1         22986 S ((WHEAT GERM) OR WHEATGERM) (3A) AGGLUTININ?
L2         12277 S 1 (6P) (SCINTILLA? OR SPA)
L3           193 S L1 (6P) (SCINTILLA? OR SPA)
L4          1148 S L1 (6P) (SUGAR)
L5            15 S L3 (6P) (SUGAR)
L6             8 S L5 AND BACTERI?
L7             7 DUP REM L6 (1 DUPLICATE REMOVED)
L8             3 S L7 AND PEPTIDOGLYCAN
L9             6 S L7 AND (?GLUCOSAMIN? OR ?GALACTOSAMIN?)
L10            6 DUP REM L9 (0 DUPLICATES REMOVED)

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L10 ANSWER 2 OF 6 USPATFULL
ACCESSION NUMBER: 2002:194709 USPATFULL
TITLE: Teichoic acid enzymes and assays
INVENTOR(S): Shinabarger, Dean L., Portage, MI, United States
Swaney, Steven M., Kalamazoo, MI, United States
Morin, Sara E., Richland, MI, United States
PATENT ASSIGNEE(S): Pharmacia & Upjohn Company, Kalamazoo, MI, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6428971	B1	20020806
APPLICATION INFO.:	US 1997-851435		19970505 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. WO 1997-US7123, filed on 5 May 1997

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-16868P	19960507 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Slobodyansky, Elizabeth
LEGAL REPRESENTATIVE: Meuting, Raasch & Gebhardt, P.A.
NUMBER OF CLAIMS: 54
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 22 Drawing Figure(s); 22 Drawing Page(s)
LINE COUNT: 1712
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention discloses a novel substrate and assay for the TAP enzyme. In addition novel DNA, proteins and peptides from genes and proteins associated with **bacterial** teichoic acid biosynthetic pathways, specifically the rodC gene and proteins and variations thereof are disclosed.

AB . . . substrate and assay for the TAP enzyme. In addition novel DNA, proteins and peptides from genes and proteins associated with **bacterial** teichoic acid biosynthetic pathways, specifically the rodC gene and proteins and variations thereof are disclosed.

SUMM The spread of antibiotic resistance in gram positive pathogenic **bacteria** is a serious problem which is only beginning to be registered in the clinic. The incidence of drug resistance is. . .

SUMM The cell wall teichoic acid pathway is found in the majority of gram positive **bacteria**, and studies with Bacillus subtilis have revealed that it is essential to cell viability. See, C. Mauel, M. Young, P.. . .

SUMM . . . the building block. Teichoic acid is a polymer of polyglycerolphosphate that is covalently attached to the peptidoglycan of gram positive **bacteria**. The enzyme CDP-Glycerol: Poly(glycerophosphate) glycerophosphotransferase catalyzes the polymerization of glycerolphosphate monomers from CDP-glycerol into a chain of polyglycerolphosphate linked via. . .

SUMM A **bacterial** DNA sequence that is capable of hybridizing to the DNA sequence of FIG. 3, under standard stringent conditions, to about. . .

SUMM . . . A collection of randomly mutated rodC genes. A selection of one or more randomly mutated rodC genes. A collection of **bacteria** having randomly mutated rodC genes. A selection of one or more **bacteria** having a random mutation selected from the collection of **bacteria**. The mutated **bacteria** selected from a mutant form of B. subtilis or S. aureus.

SUMM These plasmids may be used to create transformed **bacterial** cells and collections of mutant cells and plasmids may be easily created. So there are further descriptions of a **bacterial** cell transformed with the various disclosed plasmids and a **bacterial**

cell that is an E. coli cell, and an E. coli cell variously transformed that is of type DH10B.

SUMM A diagnostic kit utilizing the TAP enzyme and CDPglycerol to detect and monitor disease caused by gram positive **bacteria** can be created using the information disclosed herein. Following appropriate instructions from such a kit, a portion of the biological. . .

DRWD . . . Southern blot showing the DNA sequence identified as being homologous to the sequence disclosed in FIG. 3 only from the **bacteria** Staphylococcus aureus.

DRWD . . . wall teichoic acid synthesis in B. subtilis. The polyglycerolphosphate polymer of teichoic acid is linked to peptidoglycan in gram positive **bacteria**.

DETD . . . never been determined. One report describes the use of teichoic acid as a reserve phosphate source in which gram positive **bacteria** draw upon the glycerolphosphate when phosphate levels in the environment are low (Grant W D. "Cell wall teichoic acid as a reserve phosphate source in Bacillus subtilis" J **Bacteriol** (1979) vol. 137, pp. 35-43, incorporated by reference). While this role for teichoic acid cannot be disputed, the fact that. . .

DETD . . . peptidoglycan (FIG. 8). Lipoteichoic acid is a structurally related polymer that is anchored to the cell membrane of gram positive **bacteria** by the fatty acyl side chains of a phospholipid moiety (FIG. 9). Both lipoteichoic acid and cell wall teichoic acid. . .

DETD Several assays may be constructed using the TAP enzyme. Precipitation and **SPA** are two examples. Modification (alanine removal) of lipoteichoic acid resulted in improved activity of the recombinant TAP enzyme. Alanine ester. . .

DETD . . . treated sample was spotted on a GF/C filter and washed with 4.times.5 ml of 0.15 N perchloric acid before liquid **scintillation** counting. Control reactions lacking either lipoteichoic acid or CDP[.sup.3H]glycerol were included as negative controls.

DETD **Scintillation** Proximity Assay (or **SPA**)

DETD This assay is based on the ability of lectins such as **wheat germ agglutinin** (WGA) and concanavalin A (conA) to bind the **sugar** moieties present on lipoteichoic acids isolated from a variety of gram positive **bacteria**. For example, the enzyme can be mixed with buffer, [.sup.3H]CDP-glycerol, and 10 .mu.g of Enterococcus faecalis lipoteichoic acid as described for the precipitation assay above. After incubating at 37.degree. C. for 1 hour, streptavidin **SPA** beads (Amersham) containing biotinylated concanavilin A are added to the assay and the entire mix is incubated at room temperature for 30 min. in a 96 well plate. The conA::**SPA** bead conjugate will bind the radioactive lipoteichoic acid formed in the assay and the activity of the enzyme can be. . . Top Counter. A variety of lipoteichoic acids can serve as substrates and the appropriate lectin can be bound to a **SPA** bead. For example, the glucose moieties present on the lipoteichoic acids of Enterococcus faecalis, Enterococcus faecium, and Enterococcus hirae can be bound to **SPA** beads containing conA. The cell wall teichoic and lipoteichoic acids of Staphylococcus aureus containing N-**acetylglucosamine** residues can be bound to WGA beads.

DETD . . . of kits and diagnostic devises useful for the monitoring and management of disease states caused or influenced by gram positive **bacteria**.

DETD Potential uses of TAP could therefore include the diagnosis of **bacterial** infection in which **bacteria** release lipoteichoic acid into body fluids. TAP can be used to detect lipoteichoic acid in body fluids. Antibodies which target. . .

DETD . . . coli/gram positive shuttle vector pMK4 to produce pMKRODC. The pMK4 plasmid was selected because it reproduces in both gram negative **bacteria** like E. coli and it reproduces in gram positive **bacteria** like B. subtilis. Any shuttle vector of this type should be suitable. pMKRODC was electroporated into the temperature

sensitive B.. . .

DETD A shuttle vector is a plasmid that replicates in either gram negative or gram positive **bacteria**. Example shuttle vectors are pMK4, and pYL112.DELTA.119.

DETD . . . wall teichoic acid synthesis in B. subtilis. The polyglycerolphosphate polymer of teichoic acid is linked to peptidoglycan in gram positive **bacteria**. ##STR1##

CLM What is claimed is:

. . . determining teichoic acid polymerase activity in a sample, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a **bacterium** selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, and the sample to form a mixture;. . .

9. A process for determining the presence or absence of lipoteichoic acid from a **bacterium** selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis in a sample, the method comprising: combining. . .

. . . A process for screening teichoic acid polymerase inhibitors, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a **bacterium** selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, a teichoic acid polymerase encoded by DNA. . .

. . . monitoring enzymatic reactions catalyzed by teichoic acid polymerase, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a **bacterium** selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, and a teichoic acid polymerase encoded by. . .

. . . determining teichoic acid polymerase activity in a sample, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a **bacterium** selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, and the sample to form a mixture;. . .

43. A process for determining the presence or absence of lipoteichoic acid from a **bacterium** selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis in a sample, the method comprising: combining. . .

. . . A process for screening teichoic acid polymerase inhibitors, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a **bacterium** selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, a teichoic acid polymerase having an amino. . .

. . . monitoring enzymatic reactions catalyzed by teichoic acid polymerase, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a **bacterium** selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, and a teichoic acid polymerase having an. . .

L10 ANSWER 1 OF 6 USPATFULL
ACCESSION NUMBER: 2002:262214 USPATFULL
TITLE: **Bacterial** transglycosylases: assays for monitoring the activity using Lipid II substrates analogs and methods for discovering new antibiotics
INVENTOR(S): Kahne, Suzanne Walker, Princeton, NJ, United States
PATENT ASSIGNEE(S): The Trustees of Princeton University, Princeton, NJ, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6461829	B1	20021008	
APPLICATION INFO.:	US 2000-518080		20000303	(9)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 1999-122966P	19990303	(60)
	US 1999-137696P	19990604	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Leary, Louise N.
LEGAL REPRESENTATIVE: Woodcock Washburn LLP
NUMBER OF CLAIMS: 44
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 13 Drawing Figure(s); 13 Drawing Page(s)
LINE COUNT: 1344
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a direct method for monitoring **bacterial** transglycosylase activity using labeled substrates produced by chemo-enzymatic synthesis wherein the labels are selected to permit the detection of both polymeric and non-polymeric products simultaneously, either directly or following the separation of product from starting material. The invention promotes the discovery of new antibiotics with activity against **bacterial** transglycosylases by a) laying the groundwork for structural analysis of purified, active transglycosylase (which permits structure-based design); and b) providing an assay that can be used to screen for inhibitors.

TI **Bacterial** transglycosylases: assays for monitoring the activity using Lipid II substrates analogs and methods for discovering new antibiotics

AB This invention provides a direct method for monitoring **bacterial** transglycosylase activity using labeled substrates produced by chemo-enzymatic synthesis wherein the labels are selected to permit the detection of both. . . or following the separation of product from starting material. The invention promotes the discovery of new antibiotics with activity against **bacterial** transglycosylases by a) laying the groundwork for structural analysis of purified, active transglycosylase (which permits structure-based design); and b) providing. . .

SUMM The invention generally applies to an assay for monitoring **bacterial** transglycosylase activity and a method for discovering compounds like antibiotics that inhibit **bacterial** transglycosylases by screening compounds of interest for their ability to inhibit the formation of NAG-NAM (N-**acetylglucosamine** -N-acetylmuramic acid or GlcNAc-MurNAc) dimers and higher order polymers using said assay.

SUMM 2.1. **Bacterial** Enzymology

SUMM The emergence of resistance to existing antibiotics has rejuvenated interest in **bacterial** enzymology. It is hoped that detailed mechanistic and structural information about **bacterial** enzymes involved in critical biosynthetic pathways could lead to the development of new antibacterial agents. Some of the best antibiotics function by interfering with the biosynthesis of the peptidoglycan polymer that

surrounds **bacterial** cells. Because interference with peptidoglycan biosynthesis is a proven strategy for treating **bacterial** infections, all of the enzymes involved in peptidoglycan biosynthesis are potential targets for the development of new antibiotics. Although remarkable. . .

SUMM . . . (See, e.g., Gittins, J. R. et al. FEMS Microbiol. Rev. 1994, 13, 1; Bupp, K. and van Heijenoort, J. J. **Bacteriol**. 1993, 175, 1841.); second, discrete substrates for most of the downstream enzymes are either not available or not readily so. . . e.g., Pless, D. D. and Neuhaus, F. C. J. Biol. Chem. 1973, 248, 1568; van Heijenoort, Y. et al. J. **Bacteriol**. 1992, 174, 3549.).

SUMM . . . 1 illustrates the key pathways for biosynthesis of peptidoglycan. Lipid I is converted to Lipid II by the enzyme MurG (N-**acetylglucosaminyltransferase**). Several reactions occur downstream from the MurG-catalyzed reaction. After translocation, Lipid II is either conjugated to another Lipid II or. . .

SUMM . . . coupling of two Lipid II analogs, or the coupling of one Lipid II molecule to the C4 hydroxyl of an N-**acetylglucosaminyl** acceptor that is part of the growing peptidoglycan polymer.

SUMM . . . to form Lipid I and transglycosylase acts to form Lipid II. The transglycosylase and transpeptidase reactions occur extracellularly, at the **bacterial** membrane surface.

SUMM There are multiple different transglycosylases in **bacterial** cells. Both bifunctional and monofunctional enzymes have been identified (Nakagawa, J. et al. J. Biol. Chem., 1984, 259, 13937; Spratt,. . . domains of other PBPs are believed to be dependent on the presence of other proteins that have been implicated in **bacterial** cell growth or cell division (Vollmer, ibid). It is known that inhibition of transglycosylase activities, e.g., by treatment with moenomycin, leads to **bacterial** cell death. Moenomycin besides being known as antibiotic is also as an antitumor drug, see for example incorporated by reference. . . Unfortunately, there is only one membrane-free assay for transglycosylase activity. This assay involves the isolation of [.sup.14C]-radiolabeled Lipid II from **bacterial** cells or **bacterial** membrane preparations supplemented with appropriate starting materials (van Heijenoort, Y. et al. FEBS Lett., 1978, 89, 141). The isolated Lipid. . . to work for a membrane bound form of PBP1b of E. coli origin (Di Giulmi, A. M. et al. J. **Bacteriol** 1998, 180, 5652). In addition, the Lipid II substrate is difficult to isolate in significant quantities and the assay can. . .

SUMM Previous assays for **bacterial** transglycosylase activity required radiolabeling and purification of the endogenous Lipid II substrate, N-**acetylglucosamine** -.beta.-1,4-MurNAc-pentapeptide-pyrophosphoryl-undecaprenol (Brotz, H. et al. Mol Microbiol., 1998, 30, 317; Esteve-Garcia, E. et al. Poult Sci., 1997, 76, 1728; Brotz,. . . (Tokyo), 1998,. 51, 471; Mani, N. et al. J Antibiot (Tokyo) 1998, 607, 11; van Heijenoort, Y. et al. J **Bacteriol**., 1992, 174, 3549; van Heijenoort, Y. et al. J **Bacteriol**, 1992, 174, 6004). These methodologies involve multiple purification steps, yield limited amounts of Lipid II, and require radiolabel for detection. . .

SUMM . . . atoms. Heterocycle can be furyl, thienyl, imidazolyl, indolyl, pyridinyl, thiadiazolyl, thiazolyl, piperazinyl, dibenzfuranyl, dibenzthienyl, pyrimidinyl, or pyridazinyl. "R.sup.3" is a **glucosaminyl** group comprising 5 or more carbon atoms or when R.sup.3 is not a **glucosaminyl** group it can be absent or replaced by hydroxyl, oxo, bromo, fluoro, chloro, iodo, mercapto, cyano, alkylthio, carboxyl, alkoxycarbonyl, alkenyl,. . .

SUMM While the preferred sugar nucleus of the invention comprises N-**acetylglucosamine** other examples of monosaccharide units can be in either D and L configurations and can be aldose, erythrose, threose, ribose,. . . monosaccharides which represent naturally-occurring substitutions. Preferably these are deoxy sugars, fucose, rhamnose, digitoxose, preferably deoxyamino sugars such as, for example, **glucosamine**, mannosamine, **galactosamine**, aldonic,

aldaric and/or uronic acids such as, for example, gluconic acid or glucuronic acid, and more preferably deoxyacylamino sugars such as, for example, N-**acetylglucosamine**, N-acetylmuramine, N-acetylmannosamine, or N-**acetylgalactosamine**. It also is suitable to use amino acid-carrying monosaccharides and monosaccharides which carry lipid, phosphatidyl or polyol residues.

DRWD FIG. 4 illustrates the **bacterial** transglycolase reaction.

DETD . . . to catalyze the transglycosylation. Of course, transglycosylase and its homologs are derived from E. coli, H. influenzae and other gram-negative **bacteria**. Gram-positive **bacteria**, such as B. subtilis, E. faecalis, E. hirae, as well as M. tuberculosis, are also known to harbor homologs of. . .

DETD . . . be ether lipids, i.e., lipids with an alkyl (or alkenyl) group or glycolipids, i.e., lipids which contain one or more **sugar** residues. The isoprenoids, farnesyl pyrophosphate, geranyl pyrophosphate and like, as intermediates in the cholesterol biosynthetic pathway are derived from mevalonic. . .

DETD The substrate according to formula I can be radiolabeled at any suitable site, including on the GIcNAc **sugar**. Alternatively or in addition, it can be labeled on the MurNAc **sugar** with a chromophore, fluorophore, or affinity handle, such as biotin or any other binding ligand. Furthermore, the GIcNAc and the. . . attach chromophores, fluorophores, or affinity labels to the substituent attached to the lactate at the C-3 position of the MurNAc **sugar** . For example, the substituent, "A" in formula I, could be the natural peptide if the GlcNAc **sugar** is radiolabeled, or it could be the natural peptide containing a radioisotope, chromophore, fluorophore, affinity label or other group on the amino group of the lysine. Other alternative for indirect label can be a lectin, e.g. **wheat germ agglutinin**, that binds selectively to N-**acetylglucosamine** and this lectin is covalently bound to a fluorescent fluorophore like pyrene, coumarin, acridone, naphthalene, or anthracene. **Wheat germ agglutinin** labeled with fluorescein isothiocyanate can be purchased from Polysciences, Inc. of Warrington, Pa. Substitution of a radioisotope or light-emitting probe. . .

DETD . . . FRET-based assay for continuous monitoring of product. An affinity capture assay with radiometric detection can be formatted for use with **scintillation** proximity beads or plates. An affinity capture assay with fluorometric detection can be formatted to allow detection via changes in. . .

DETD . . . detection. The Lipid I substrate analogs are separately converted to the corresponding Lipid II analogs by MurG-catalyzed transfer of N-acetyl **glucosamine**. The GlcNAc utilized for transfer to the non-biotinylated Lipid I substrate is radiolabeled. Following conversion to product, the MurG is. . .

DETD . . . labeled Lipid I substrate analogs are separately converted to the corresponding Lipid II analogs by MurG catalyzed transfer of N-acetyl **glucosamine**. Conversion to product is detected by an increase in emission of the acceptor when the sample is irradiated at the. . .

DETD . . . the lysine side chain. The substrate analog is converted to a Lipid II substrate analog by MurG-catalyzed transfer of N-acetyl **glucosamine**. Subsequently, transglycosylase is added to the Lipid II analog. The reaction is quenched at intervals and aliquots of the reaction. . .

DETD . . . assay allows one skilled in the art to screen and identify transglycosylase inhibitory activity of known and unknown antibiotics affecting **bacterial** wall synthesis. Specifically they comprise vacomycin, teicoplanin, ramoplanin, paldimycin, DuP 721 and DuP 105, methicillin and gentamicin, oxazolidinones, A/16686, A/16686. . . produced by genetic engineering or by semisynthetic chemistry, are useful in the prevention or therapy of infections caused by antibiotic-resistant **bacteria** in humans and animals. Not only

all presently known lantibiotics: nisin, nisin Z, subtilin, epidermin, gallidermin, pep 5, duramycin and. . .

DETD Ramoplanin (FIG. 8) is a cyclic glycolipodepsipeptide antibiotic that kills gram positive **bacteria** by inhibiting cell wall biosynthesis. Ramoplanin blocks the conversion of Lipid I to Lipid II, a reaction that is catalyzed. . . by this inventor to inhibit the polymerization of Lipid II; therefore another mechanism is discovered by which ramoplanin can kill **bacterial** cells through inhibition of the transglycosylation step of peptidoglycan synthesis. Using a synthetic analogue of Lipid II, the evidence is. . . to self-associate to form observable fibrils. The mechanism of action of ramoplanin has been investigated in the past in permeabilized **bacterial** cells and membrane preparations by following the incorporation of radiolabel from a precursor into various intermediates along the pathway to. . . proposed to act by completing substrates required for peptidoglycan synthesist. Unfortunately, the prior art difficulties in isolating Lipid intermediates from **bacterial** cells have hindered studies of their interactions with ramoplanin. Moreover, the natural lipid intermediates contain a long 55 carbon polyprenol. . .

DETD . . . II derivatized with biotin is synthesized from UDP-GlucNAc and UDP-MurNAc-pentapeptide (conjugated with biotin on the amino group of lysine), using **bacterial** membrane preparations. Biotin can be attached by a crosslinking agent or linker. Linker can be selected from any of following:. . .

DETD FIG. 7 discloses in vitro polymerization of biotinylated-[.sup.14C]-Lipid II accomplished by incubation with **bacterial** membrane protein, and product is detected by ascending chromatography in isobutyric acid: 1M NH40H (5:3). The product is the result. . .

DETD In FIG. 7, biotinylated Lipid II serves as a substrate for transglycosylase present in **bacterial** membranes. Control: incubation of **bacterial** membranes with biotinylated Lipid II, which is also labeled with [.sup.14C] GlucNAc, demonstrates conversion of substrate into peptidoglycan product that remains at the origin (Rf=0). The curve labeled "0.06 .mu.g/ml Moenomycin" illustrates incubation of **bacterial** membranes with biotinylated Lipid II, which is also labeled with [.sup.14C]-GlucNAc, in the presence of the known transglycosylase inhibitor moenomycin,. . . data demonstrate that the product obtained in the absence of moenomycin is peptidoglycan. It is thus clear that any other **bacterial** cell wall inhibitor can be equally screened.

DETD Sources of transglycosylase include a) **bacterial** membranes prepared following lysis of **bacterial** cells, b) normal or regenerating **bacterial** spheroplasts or protoplasts from which the cell wall has been removed, c) **bacterial** cells permeabilized with organic solvents, d) mutant **bacterial** cells containing defects in the outer membrane that render them permeable, or e) transglycosylase enriched or purified from **bacterial** membranes or lysates. **Bacterial** sources include laboratory strains, clinical isolates, and derivatives thereof which have been genetically engineered to express specific transglycosylases in membrane. . .

CLM What is claimed is:

. . . atom, "R.sup.2" is a substituted or unsubstituted alkyl or alkenyl group comprising at least five carbon atoms, "R.sup.3" is a **glucosaminyl** group comprising at least five carbon atoms, "A" is a substituted or unsubstituted amino acid residue or a peptide comprising. . .

7. The substance of claim 1 in which "R.sup.3" is an N-**acetylglucosaminyl** group.

. . . atom, "R.sup.2" is a substituted or unsubstituted alkyl or alkenyl group comprising at least five carbon atoms, "R.sup.3" is a **glucosaminyl** group comprising at least five carbon atoms, "A" is

a substituted or unsubstituted amino acid residue or a peptide comprising. . .

. . . The method of claim 22 in which at least a portion of said sample comprises a portion of a lysed **bacterial** culture, a portion of a supernatant thereof, a portion of a membrane fraction thereof, a portion of a protein fraction. . .

. . . atom, "R.sup.2" is a substituted or unsubstituted alkyl or alkenyl group comprising at least five carbon atoms, "R.sup.3" is a **glucosaminyl** group comprising at least five carbon atoms, "A" is a substituted or unsubstituted amino acid residue or a peptide comprising. . .

. . . atom, "R.sup.2" is a substituted or unsubstituted alkyl or alkenyl group comprising at least five carbon atoms, "R.sup.3" is a **glucosaminyl** group comprising at least five carbon atoms, "A" is a substituted or unsubstituted amino acid residue or a peptide comprising. . .

. . . atoms, an aromatic or heteroaromatic group comprising 3 to about 55 carbon atoms and pyrophosphate protecting groups, "R.sup.3" is a **glucosaminyl** group comprising at least five carbon atoms, "A" is a substituted or unsubstituted amino acid residue or a peptide comprising. . .